

Transmission of F+.

Jan 21, 1952

Clear Supernatant (2 antif.) of 58-161 = A ~~ladd~~ Activity: ca: 3×10^6

- A.
1. A + W1177 (ca 10^7 /ml) + sm 12 - 2:30 Inc.
 2. A + W1177 v. little
Refrigerate
 3. 58-161 cells ca 10^7 /ml + W1177¹⁰ + sm in Pernassay Inc. { considerable
 4. " " 10^8 /ml + W1177 + sm " Inc. } growth.

Streak out EMB lac after 2½ hours incubation.

- 1: Ca 1% +
2: No +
4: Ca .1% +

Pool 20, lac+ colonies as inoculum for crossing test.

For 'series, pick as many colonies as possible.

Check all inocula by streak on EMB lac*

A1'	-	✓
A1'	++	✓
A3'	++	✓
A3'	+++	✓
A4'	+++	✓
A4'	+++	✓

Note A1' alone: - ✓✓

Cells are evidently very efficient in transfer of F+

(washed cells)

- B 1/24/52. W1177 ca 10^8 + 58-161 in Pernassay A B C
1. 58-161 ca 10^7
 2. 58-161 ca 10^8
 3. 58-161 supernatant (ca 10^8 cells)
 4. 58-161 ca 10^8 Refrigerate in Pernassay. - - -
- { Incubate 2 days ++ ++

A. 2 cols { W1177 Reisolated and tested.
 $\frac{1}{10}$ cols C ∞ cols

1/26. All plates bare except 2B, 2C.

∴ 10^8 cells transfer ca 10% F+ in 1 hour. But atest 2A. F+ may not be allowing at first growth!

Cross each x W1607 (unwashed)

Antibiotic: 2B:+++ 2A, 1C, 3C, 4C: -

2 colonies: - probably contaminants

Sources of F+?

902

Jan. 21, 1952.

F+ may be produced by other bacteria, futile and infertile. Test by growing propagules overnight in broth with W1177, followed by streaking out on E17B to separate W1177 component. Cross extracted W1177 with W1607 for F+ test.

1 w^g/l K12

2

(3) 3

4 4

5 5

6 6

7 7

8 8

9 9

10 10

11 31

12 B

13 ML

14 W1811

KO

1 16

2 17

3 18

4 19

5 20

15 W1305

21 S. gallinarum

22 pullorum

23 LT2

24 LT22

25 LT7

26 coli 1

27 coli 2

28 flora

29 coli 5

30 coli 4

31 coli 3

W1177 found ✓

2-3 cols.

ca 1/2 Lect. Recol. ✓

✓

F status of W1177 (xW1607)

+++

-

+

-

W1177

Latex -

(Mal)

- ✓ -

-

✓

✓

✓

90%

-

✓

✓

1/2

✓

Latex

Mal - Mammal cols ✓
- also SR.

APF

Latex impure

Recol. (severe)

+ ? Recol.

1 col. Recol.

+++

(W-1551-5) 1553

16 17

18 19

20 21

22 23

24 25

26 27

28 29

30 31

31 32

✓

✓

1/2

<10

1/10

✓

few cols. Recol. ✓

1/3

✓

1/2

✓

✓

v. few Recol. ✓

-

-

-

-

+++ -

-

-

-

-

-

-

-

-

M14 Broth molts in 10 ml Penassay 10:45 AM 1/22.

Add sm 10 µg/ml 4:30 PM. Streak out ① 9PM. Replate to col or Mal - next day.

Transmissible F+ seems to be confined to K-12. Infertile strains are evidently not restricted by this mechanism. Except?

#14 again scored Lac+ on streaks although care was taken to pick only Lac- colonies!

Replicate from same plate. Streak out Lac+ streak test.

Sources of F+?

902a

			suspensions of W1177	x W1607	
32	wg 6			++ ✓	Reacts suspension for A2 test.
33	wg 7	tac- "peculiar"	++	T -	
34	wg 8	later		+++ ✓	
35	11			+++ ✓	
36	12		+	+++ ✓	
37	13		-	T why? ✓	check - 37 is lac- prototroph; wg 13 is lac+ prot.
38	14			+++ ✓	of possible cast.
39	15			- ✓	
40	16			+++ ✓	Prototroph not found or repeat
41	17		+	3? ✓	
42	18			-	
43	19			-	
44	20	all Lac+	-		
45	21		++	T	
46	22		++	✓ - sci! ✓	46 is lac+ auxotroph but wg 22 is similar
47	23			-	
48	24			1? ✗	
49	25			1? ✗	
50	26		++	T	
51	27			T	
52	28	lateral also -	++	T	
53	29			-	
54	30			1? ✗	

Reactions: all +++ (W1607) above EMB Lac specific

3B
14B*

18B EMB lac: all + Pick + streak from EMB lac sm.

18A2: Replic from same plate →
14A2 (Replic W1177 colonies from same plate as 14A1) → + → . W1811 = F+

14A1 (See above): Mixture of lac+, lac-: evidently not clearly picked.

* When streaked out on EMB sm, the mixed culture of W1811 + W1177 showed plasmid in the thick streak. Origin of phage?

WG-16 also found to be lysogenic on same basis.

Conformation of F+ transfers from other strains

9036

Jan. 28, 1952.

A) The following wg x are fairly clearly F+ :

wg: 1, 6, 11, 12, 14, 16, 21

B) The following are uncertain F+

wg 3, 17, w1553

C) The following were inadequately tested

wg 13, 21, (22), 26, 31, 38, 20

Note: wg 22 is auxotrophic.
A ^{Mat-} lac- prototroph was
obtained from wg 13 + w1177's
selection

B: Repeater verify with w1177

C: Repeat transfer of F+ to w1607 for comparison of F+ agent
number follows wg.

B	36	+	?	
	37	+	+	- def.
	41	±	+	+
	44	±	—	—
	46	—	—	—
	50	—	—	—
	51	—	—	—
	52	all -	—	—

Repeat 36, 41, 44

C. F(wg x) to w1607: 6, 11, 12, 14, 16 Label w1607 (Fwg 6...)
Note: D12, D14 OK; D6, 11, 12 not F+ on first test See 908

D Transfer F+ to w1177: wg 6, 11, 12, 14, 16 ~~38~~ ~~41~~ ~~44~~ 17 "D44" ~~not OK~~

E Test Kauffmann's O Types (w1551-) for F+ to w1177. Label pro-type
Reults 3, 6-12. all F-

F+ transfectus from wgx

902c

February 19, 1952.

(memo) all recent attempts to repeat F+ transfectus from wgx have failed. Technique consisted of bursting or sonication for 1 min of wgx. Are these F+ perhaps sensitive to son?

Repeat 902-c-d by two methods: (a) without sonication from mixed culture to EMB lac (b) by microculture & splitting on EMB son.

Proc:
D12
D14

Test from
washed
cultures

a: C16 D16 C11 D11 mass culture gone ~~at~~ F+ proceed to isolation from single col.

C12, C14 turbid or opaque. Resoliate

C-D 3, 6, no signs of F+.

b. C-D 3, 6 → C3. 1 lyzed? colony nodif (lac-). No peculiarity at neutrals no F+

C11 F+ (wells)
C12 F+ (wells)
C14 F+ (wells)
C16 OK. F+

Isolate F+ from a, b as follows

D11 F+ strong
D16 no F+

C11 a D11 a
C12 b
C14 b
C16 a D16 a

Try 3 and 6 again

C, D11, 16 single colonies F- by pul repl. plate test.

Recheck 2 single colonies via broth tubes.

A/B	C3	C6	D6	D3	D16	C21	P21	C17
D16	--	-	-	-	-	-	-	-
D11	+, ++	✓	-	-	-	-	-	-
C14	±, +	✓	-	-	-	-	-	-
C12	±, ±	/	-	-	-	-	-	-
C11	++, +	/	-	-	-	-	-	-

F+ transfers from wgx.

902cc

March 16, 1952.

A) The following are F+ transduced, but single colonies not yet recovered as F+ :

D3 : none in pool.
S.col.
C21 : 2 cols in #1, none ~~in~~ ?
D21 no F+ even in ~~one~~ pool
C17 " " " "#4 pool?
D35 - ✓ single colonies: 8/8 F-?

tests passing: old water ~~some~~ F+ were stable?

B) Not yet transduced even in mass culture:

C3	✓	x	#1,2,4,6/8
C16	✗	✗	
D16	✓	✗	#1/8 1 pool x
C6	x		
D36	x		
C17	x		

C) Not yet attempted:

C35 - 1 colony is F+ kind of pool!
x x

D) accomplished See 923

C-D 11
C-D 12
C-D 14
D 17
D 35
C 21 ?

Can wq x acquire F+.

1/26/52

1. Y18

2. WG-3

3. WG-4

4. WG-31

5. WG-24 (W1177 F+)

Grow progenies in 1 ml Pernasoy overnight. Mix with

W1817 12N26. - A27

Strain on EMB Lac, (A)
resist Lact. Grow these with W1177. Reciprocate W1177
(B) and test x W1607. Strain 1/29

6	903-F1
7	" 1
8	" 2

A6-903

Results

Note: #2 +ve/green - in first step

#8 ~~+++~~ W1817 carried along with Lac in second step.
Save 903 A6-8 for futurity test

B.

1	++	
3	++	Lac-
4	++	Lac-
5	turbid	
6	+++	
7	+++	

If reliable, this would indicate that
wg 3-4-31 could become F+ if not so
already.

A. 1. (x W1607) - ! Results. (cf. B1) Strains, x W1607, F+

B

1	+++	
3	+++	wg 4
4	+++	wg 31
5	-	

} can become F+ on contact if not already.

∴ WG-24 is F-, remains so

Repeat: Compare original WG-4, WG-31 as sources of ~~F+~~ vs. F+ donor.
= C = D

To W1177

✓ WG-4, 31 originals do not donate F+ to W1177 (v x 1607)

WG-4, WG-31 after exposure to W1817 become F+ donors.

W1177 (WG-4)	C	x W1607
(WG-31)	D	-
	C	++
	D	+++

Interactions of wg x F+.

9036

February 10, 1952

Cultures from 902. C are W1607 F+ D are W1177 F+. (all 902)

1	C 6	x	D 6	- -
2	C 6	x	1177	- -
3	C 11	x	D 11	- -
4	C 11	x	1177	- -
5	C 12	x	D 12	++ ++
6	C 12	x	1177	- -
7	C 14	x	D 14	++ ++
8	C 14	x	1177	- -
9	C 16	x	D 16	- -
10	C 16	x	1177	- -
11	W1607	x	D 6	- -
12	"	x	D 11	- -
13	"	x	D 12	++ ++
14	"	x	D 14	++ ++
15	"	x	D 16	- Col?

16	58-161	x	W1177	
17	A	"	3	
18	B	"	+++	
19	C	"	2	
20	D	"	++	

A = aerated overnight B = revo 10²⁰ c.f.u. 3 hours
 C " " 6½ hours
 D " " 6½ hours
 # cultures harvested at 11 AM; 1:15 PM; 4:45 PM
 and refrigerated to 5 PM for plating

Reisolate W1607, W1177(F+, wg x)

W1808	x	1177	+++	
1809		1817	+	
		1177	-	
		1817	+	3 of 1809 F+.

W1611 x	1607	{ turbid
1875		±
1177		±
1876		+

behaves like a partial F-

wg ⁴	W1611	161	++	F statics?	1705	1607 {	-	W1145	1607 {	turbid
		1607	Col						1875 {	+
		1177	-							
		1817	-							

1611 ++

1611 -

Segregation & role of F+
in outcrosses

904

January 26, 1952

1/27
AM

			1/28
A	W1446 wg 4	x W1607	
B	"	W1816	T
C	W1451 wg 3	W1607	++
D	"	W1816	12
E	1446	x 58-161	18
F	"	x W1830	28
G	1451	x 58-161	6
H	"	x W1830	40

903E1
status of W1830
is still doubtful.

Applying ~~to~~ away as fresh D(0).

In view of 903-3, "segregation" would not be meaningful
(Unless F+ can not be transmitted extracellularly or minimally.)

W1868 (wg 12) x 1808 . 31 +++
 1607 . 1- +++
 1875 . 1+ +++
 1451 . 3 +

W1808 (wg 31) 1451 . 3 ++
 1878 . 21 +++

W1811 Sterility: Summary & Expts.
Lysogenicity

905

1/28/52

In course of 902-14A, plaques were noted in W1177 that had been grown with W1811. The lysogenicity of W1811/W1177 was confirmed. The plaques are readily seen on sugar agar (supressing W1811 bacterial growth.) Check other past-cultures from Maes! All of his strains proved to be lysogenic on W1177. Their history is given as K12 $\xrightarrow{(+)}$ KIT $\xrightarrow{\text{out about.}}$ Presumably phage entered (mutated?) at * + + Some KIT series is futile, the phage is not related to W1811 sterility.

1/28 Grow W1811 c and s 58-161, W1177.

1	58-161 + (W1177-W1811)	+	W1811 sterility is not absolutely transduced in mixed cultures
2	(58-111-W1811) + W1177	++	
3	() + ()	++	
4	58-111 + W1177 + W1811.	+	
5	See 891 for control, 902-14B.	+++	

2/1/52 Further work on this phage assumed by E77L. W1811 has been verified as F+ (902). Its phage acts equally on W1177, W1817.

1811 x 1451	wg 3	<u>cont?</u>
1611	v	-
1607	1-	=
1868	12	
1808	31	1? 5 colonies: Repeat \rightarrow 4 colonies
1205	wg 29	
1145	wg 2	

W1678 - aeration effects and F character 906

1/29/52.

1/26/52: 1678A x 1607 1816 ~~F+~~ ++ F- !

Repeat 1/29-30/52.

1/30

	1/31	2/1
1 W1678 x W1607	+++	✓
2 " " W1816	++	✓
3 " " W1177	++	++
4 " " W1817	+	9 cds.
5 W1678aer 1607	++	—
6 " " 1816	±	+
7 " " 1177	++	++
8 " " 1817	—	—

1678
1817
1177

1830
1830 900 E
1830

All 907

: If aeration has any effect on W1678, it is to decrease its fertility with F+. So W1678 a "super" F+.

58-161

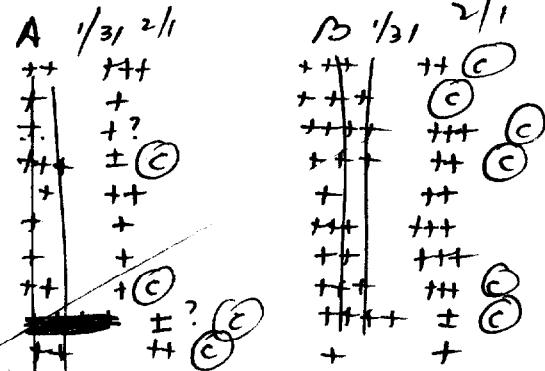
January 30, 1952
Temp. Vessel Medium...

1	37	10 ml tube no aer	X	1177	A
2	"	Plate - liquid	X	1817	B
3	"	tube aer			
4	"	Plate - agar			
5	" 30°	tube no aer			
6	"	" aer			
7	"	plate 11g.			
8	"	plate agar			
9	44°	10 ml tube agar			
10	"	plate 11g. v. poor growth			

A

As in previous expts., adjust approx.
for cell density.

Note very high yields of 44° cells!

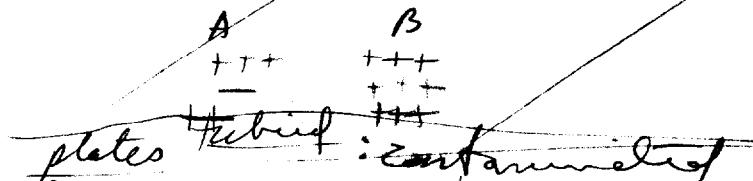


Many of these plates show
contamination: (C)

Repeat comparisons of 30°, 37° (30° thermostated).

58-161

1	37°	-
2	30°	-
3	30°	-
4	30°	-



B

These results all N.G. owing to contamination

C

Growth conditions and F- phenology
of various strains

2/1/52

	Temp.	Aeration	58-161
1	37	+	
2		-	
3	30	+	
4		-	
5	1678-37°	+	
6		+	
7		+	
8		+	
9	1678-37°	-	
10		-	
11		-	
12	1678	-	
13	1678	+	
14		+	
15		-	
16		-	
17	1816	.	
18	1816A	.	
19	1816	.	
20	1816A	.	
21	1817	.	
22	1817A	1177	
23	1817A		
24			

		x 161 1677	4 — (2 col/s.)	x W1817	B. — (3+ 4 col/s.)
x					
6		1607			+++
7		1816		-	++
8		1177		+	++
9		1817		-	-
10		1607		++	+++
11		1816		+	++
12		1177		++	+++
13		1817		+	4
14		58-161		:	5
15		58-161 A		++	++
16		58-161		:	40
17		58-161 A		++	+++
18					
19					
20					
21					
22					
23					
24					

+++ (contains.)

Does 1816
respond to
aeration?

(aeration was
interrupted).

W1817 crosses
if fully aminated throughout
except the prototrophic
1 end. 1677

2/2/52	23	161	W1817	-	++	
	24	161	W1817	++	+++	
	25	161A 37°	1177	+	++	
	26	161A 37°	1817	++	++	
	27	161A 26°	1177	+	+	
	28	161A 26°	1817	++	+++	
	29	161A, Plate	37	1177	5	
	30	161 "	37	1817	+	

{ ?

2/1	31	161 44°	1177		++	
	32	"	1817		++	
	33	"	1177 44°		++	

∴ 44° does
not produce F-
nor manipulable
fertility.

Conditions of aeration for F- phageology

9076

February 4, 1952.

(Growth ass)	1 58-161 37°	A x W1177A	B x W1177	C = W1177	
2	" aer	8	++	++	
3	" 26°	-	+++	+	
4	" aer.	-	++++		
5	W1816	x W1177A	++		
6	W1816 A	x W1177A	+		{ demand.
7	W1816 A	x W1817	++		
8	W1817	x W1607 A	+++		{ demand.
9	W1817 A	x W1607 A	++		
10	W1817 A	x W1816	-		
11	161 37°	heavily grown	++		
12	161 A	heavy	+		
13	"	B Penicillin	±		{ delete media
14	"	26°/10 " heavy	-		
15	" 26°	-	++	++	work!
16	161 A. Reinc 10 AM from 11	-	++		all cells stand upright to 8P7
17	" 12	-	+		
already heavy	18	continued to	-		
heavy.	19	161 removed 2PM - 5P11	-		
	20	17 "	++		Moderate growth = unsaturated
		"	+		saturation

1. low temperature; dilute media do not mitigate aeration effect. Phase of culture cycle? (cf. 19-20 vs. 11; 16, 17)

Suggested experiment: dilute cells to saturation. Assay.

Dil 9072 remoulte and assay at partial, complete saturation.

A = aerated overnight B = remoulte, aer ~~no~~ home

C " "

D = rem ~~no~~ no aer.

21 58-161 x W1177
22 " A
23 " B
24 " C
25 " D

Cells harvested at 10³⁰ AM; , inf. to plating at PM.

aeration effect

907c

2/10/52

A are aerated overnight ($\frac{2/9 - 2/10}{SPM - 12M}$).

1	58-161 x W1177	+	+
2	" A x "	-	-
3	909-1 x W1177	+	+
4	909-1 x W909-4	++	++
5	909-4 x W1607	+++	+++
6	W1607 x W1177	-	-
7	909-1A x W1177	-	-
8	909-4A x W1607	-	10 col.
9	909-1A x 909-4A	-	-
	909-1 pur x W1177	++	
	909-4 pur x W1607	+++	

2/13.

11	1875 x 1177	++	++
12	1875 x 1876 1876	+	++
13	1876 x 1607	+++	large, more numerous than 12, 21
14	1607 x 1177	-	-
15	1875A x 1177	±	+ { little effect of aeration.
16	1876A x 1607	±	+
17	1875A x 1876A	±	8
18	58-161A x 1177	-	1
19	58-161 x 1177	++	++
20	58-161B x 1177	-	6

$$B = 58-161A + \text{Pennesay} \quad 4:30 - 6$$

. See 908-~~15, 16~~

∴ no recovery in this interval.

2/15

21	58-161	x 1177	+	1
22	58-161A	x 1177	÷	
23	58-161B	x 1177	+	

B = 58-161 algal rot suspended in supernatant of 58-161A for 2 hours, & re-suspended.

∴ 58-161A supernatant had no effect on 58-161

Transfer of F^+ .

2/1/52

ca. 10^9 each cell type in ~~2 flasks~~ 3 ml. $3^{30} - 4^{30}$ PM.

			XW1607
1	SD-161 + W1177	bre., Pernassay	+++
2	SD-161A + "	" "	+++
3	SD-161 + W1177	perf. "	-
4	W1678 + "	bre. "	+++
5	W1678A + "	bre. "	+++
6	SD-161 + W1177	bre. D(0)	-
7	" "	bre. D(0) + MTLB,	-

For assays pool 2 (A) and 10 different (B) W1177 isolates.

XW1607. 2/6 A and B agreed in each case.

F^+ is transferred in Pernassay but not in synthetic medium under comparable conditions. This agrees with behavior of three-way crosses. Try growth in synthetic for longer periods. Aeration also seems to be necessary. Aerated cells, presumably phenotypically F^- , still transfer F^+ .

8 SD-161 + W1177 in D(TLB, BM) $\frac{1}{P}$ aerated. 3 overnight.

9. Isolate W1177 by streaking out and via sm.

The labels on 8, 9 were unfortunately lost. What was probably 8 failed to show transfer; in 9, 10/14 isolates were F^+ .
Repeat experiment 2/11/52 (now 10:20 AM) 510 PM

8 1 F^+ / 15 tot
9 5 F^+ / 12 tot.

also streak after overnight. 8-9A. 1st Replicates n.s.

Transmission of F+

Feb. 12, 1952

11 12 sm 1000/ml.

—

13 heat 56° 30m.

14 boil 5m

1 colony ?

It has been previously established that W1811 washed cells do not evoke fertility in $\text{W1607} \times \text{W1177}$ on D/V agar.

Add ca 2×10^9 cells W1811 1ml to 1ml Penassay + ca 10^9 W1607.

Treat tubes as indicated. Incubate together from 12³⁰ - 2PM.

(Put heat W1811 for heat experiments etc...). Rewash. Plate with W1177

This transfer technique; assay n.g. Phenotypic delay in F+ ??

15 58-161 + W1607 in Remassay

16 58-161A + W1607 " " of 907 20.

4st single colonies. Replic. tests n.g.

Phenotypic lag + F+ transmission:

February 16, 1952. W1305 \Rightarrow W1177.

ca 10^9 /ml in Penassay 37° 2PM - 3³⁰

$\times 1607$ immmed. Single W1177 survt.

21 W1177 in Penassay

22 (W1177 + W1305) in Penassay

23 W1177 original

24 W1177 (Pen.) + W1305.

+++

-

-

1305 controls all -.

Phenotypic delay with F+ is not

borne out by this experiment.

Reactions 7/13

9058

2/19/52.

W1305 from 2 TSA plates to 10 ml. 1 ml to 10 Penassay. Let stand
ca 10°/ml each.

- 1 No treatment. Incubate together 3PM - 5³⁰
- 2 Boil 5 min.
- 3 Heat 58° 30 min.

~~x~~ W1607 | 2 and 3 sterile
1 ca 21305: 1 117

1. showed many prototrophs; 2 and 3 - ?

but plates were contaminated!

Repeat.

- 4 Control
- 5 Heat 58° 30m.

1305 + W1607.

^x W1177.
++ contain?

W1305: 1 ml sterile

W1305 stock? or
plates contam.)

F+ from different sources.

909

January February 1, 1952.

Mix routine: Grow overnight. Shake out on EMB lac; EMB lac sm
 Retracts from son to EMB lac \rightarrow
 pure cultures. Pick from 1-5 cultures for pool
 for initial tests. Not s.c. pure!
 ✓ by crossing to W1177 or W1607 resp all now +

		x			
1	2	1177	++	++	
2	2	5	++	+	
3	2	6	++	+	
4	2	1678	+++	++	
5	3	1177	±	++	
6	3	5	±	+	
7	3	6	+	+	
8	3	1678	++	++	
9	5	58-161	+	+	
10	5	1607	++	++++	
11	5	1678	-	4 cols	
12	6	58-161	+	++	
13	6	1607	++	+++	
14	6	1678	-	2 cols	
15	1678	1607	++	+++	
16	1678	1177	++	++	
17	1678	58-161	++	+	
18	1	4	+	+	
19	1	1678	++	++	
20	4	1678	+	6 cols	

Summary. $W1678 \times \text{+++} \quad F- \quad F^{161} \quad F^{1678} \quad F^{1177} \quad F^{58-161} \quad (BM4 \text{ fert})$

$1607(1678) \quad ++ + \quad +$

$1607(58-161) \quad +++ + \quad +$

Note 1×4 . Compare

$1 \times W1177$
$4 \times W1607$
1×4
1177×1607

$\therefore W1678$ is relatively infertile
 with $F+$ either derived from 58-161
 or W-1678 or K-12.

$F-$ reinforced with $F+$ from various
 sources behave in the same way.
 There is no evidence that $W1178$
 carries a different F , but the
 infertility of $F+ \times F+$ is emphasized
 especially in $\times W1177 F+$.

2/5/52.

Brown 08161 30° 3 TSH plates. Harvest, wash saline, dry over night
2/5/52 Extract H₂O ca 5 ml. → A) sup. B) sediment

Extract B with 1% saline overnight refrigerate.

Add 1 ml supernatant + 1 ml 10⁹/ml W1177 to 5 ml Psm.

Inc 5:05 PM - 8 PM streak out on EMB bac

2/6/52 No lact colonies seen. Pick individual and pooled colonies

① Replica xx test from EMB streaks

② Test 4 single colonies, and ca 40 pooled colonies.
x w1607.

all F- Ende extract: ~~no~~ no transmission

8/11

Incubation effects

Feby. 19, 1952

3. Harvest 58-161 from 2 T5 Agarates. Ca 3/4 of this into 10 ml
fresh Pen-assay (~~—~~ & 10^6 cells/ml) dilute 12:15 - (3-4 PM)
= B.

1. 58-161 $\times W1177$
2. 58-161 A plate contains
3. " B + $\times 1876$ +

Repeat: dilute in Penassay experiment of 58-161A = B.

- | | |
|--|---|
| ✓ 4 58-161 B
✓ 5 58-161 A
✓ 6 " + W1305 in Penassay. (1 ml 58-161 $\frac{ca 10^6}{12.25}$ cells/ml.
✓ 7 " — " " " | $\left. \begin{matrix} \\ \\ \end{matrix} \right\} \times W477$ —
carbon
contains.
++
+++ |
| 8 58-161 | |

Compare transfer of F+

912

~~Transfer~~

2/17/52.

	W1177 W1177	in Peassay	Ca. 10^8 /ml each.
1.	SS-161 + W1177	"	
2	" "	acetate	12:50 - 2P

3 SS-161A + ~~W1177~~

student and test isol col.

1 2+1/8
2 0 1/2
3 2+1/2

∴ SS-161A donates F+ acetate may inhibit transfer

1 1/2 hr. mixt Peassay

1	SS-161A	X W1177 (1177)	-	
2	" + W1305	2		
3	" + W1305A	1		
4	" + W1811	6		
5	" + W1811A	4		
6	" + W1607	5		
7	SS-161 + W1607	18		
8	SS-161A - incubated	2		
9	W1607 + W1305	-		
10	" + " A	-		
11	" W1811	-		
12	" " A	-		
13	1305 W1811	no X	-	
14				

From 4 and 5, still F+ may have stimulated SS-161A but exchange even to W1607 was limited. Note: W1607 was aerated!
To Do's Compare 1607A; 1607 as receptors of F+ from W1305.
But note also low yields on 7.

Structure of diploid

2/19/52.

W112 x W1435

lac I_b - lac I_a - V₆^R Het.

EM5 bac.
D10) → EM5 bac } ca 15 plates
ca 100-200/plate.

3 lac+ found.

4+ in second set

4 in 3d.

1 lac++
2 lac + slow
3 lac++

V₆
R S =
S R = 1.

~~ext. + 2. rec. 1.~~
4R 4TS

H304 4 lac+ -

S RS

lac-st I_b lac-st I_a

5 lac++

S

6 ++

S

7 ++

S

8 ++

S

9 ++

R

H305 10 lac+ -

S

11 ++

R

W112

R

W1435

S

Test segregants (mass streaking) of H-304-5.

H304 6 lac+ all S 5 lac- all R

305 7 lac+ all S 5 lac- all S sic!

H304 is therefore presumably A; H305 may be B.
(may have arisen by mutation in a

B: or { } A + - !)

If so, the lac- of H304 should be stable, i.e. A-B-V₆^R
of H305 should be unstable A-B+V₆^S.

Induce the mutation of these - (pool'd) and compare with parents.

Re-streak single colonies, test +/+, 1- from each.

T6

H304 7lac- 15 6R 8lac+ all S
H305 9lac- all S 9lac+ all S

This confirms
previous page.

Check pooled lac- for mutability.
of parents

	48h. EMB lac	
H304	M	V ₆ R
H305	S	V ₆ S
W143	M	V ₆ R
W112	S	V ₆ S
Lac+		V ₆ S

∴ These two lac- component
appear to be parental, not
complementary crossovers.

X++	S	+	+
X--	R	-	-
P1	R	— ^m	+
P2	S	+ X - R	
	V ₆	A	B

$$H304 = \frac{X++}{P1}$$

$$H305 = \frac{X++}{P2}$$

Repeat cross 3/13/52 8 plates EMB lac ^{x 400} 1? lac+ ✓
9/13-12 slow? lac+ 1- saved as H308

3/17/52. 4 x 600

2 lac+ 9/13:13-14

13: probably lac_v, sum. 12

3/27/52. ¹⁴ lac+ 8 x 400 cols. 4 Lac+ ^{+ v?} ^{few + ing?} 15, 16, 17, 18.
9 x 300

D(smr) crosses re +.

9141

February 22, 1952.

Closest comparison would be $W1368^{+R} \times W677^{-S}$
 $\times W677^{+S} F+$. Father is being
 purged.

2/22. Crosses in D(0) ± smr.

1. $58-161^{+S} \times W1177^{-R}$
2. $W1368^{+R} \times W677^{-S}$
3. $W1802^{-S} \times W1876^{+R}$
4. $58-161^{+S} \times W1876^{+R}$
- (5) $W1875^{+R} \times W677^{-S}$

D(0) D(smr)

++	+	=] agrees with Hayes
+++	-	=	
+++	-		
?	-		
+++	-		

2/25

- 11 1368^{+R} 677^{-S}
- 12 1368^{+R} $677^{+S} F+$
- 13 1607^{-R} $677^{+S} F+$

: $\frac{4}{2}$ SM
 $\frac{1}{2} \pm$

$\frac{3}{2}$ SM +++ | -
 $\frac{3}{2} \pm$
 $\frac{1}{2} \pm$

| (1-4): ± (14 cols.)

2/26

- 14 1802^{-S} 1876^{+R}
- 15 $58-161$ 1876

F₁ purged.
 ✓

∴ Hayes' observations are again confirmed, ^{Also} $677 F+$ becomes competent on smager, and the sm effect is related to compatibility.

$677 F_1$ - same $F+$
 $\frac{F+}{\text{use } F_1 \text{ traits}}$
 $= W1896$

- 16 1895 1177 0 SM
- 17 " 1896 ++ +
- 18 1811 $W1177$ - -
- 19 1678 1177 +++ ++
- 20 1678 1876 1 col. -

note near sterility $F+ \times F+$

Repeat a). (old 1368 smr.)

b)

Result not confirmed!

- 11 0 -
- 12 ++ -
- 13 + -
- 14 +++ -
- 15 ++ - ?

0 SM
 1 col? " 3:
 8 " " -
 1 col " -
 ++ - !

Try 1678 S^R

3/10/52. W1368 x W677 D(0) D(smr)
 ++ 0, 0

x W1896 + 2, 1

combinations upheld F+ and resistance to smut, but not stability.

3/12/52. Repeated:
 W1368 x 677 D(0) D(smr)
 +++ 0
 1896 + 3

again. Also note greater compatibility of F+ x F-.

The survival factors may be due in part to the loss factors of S^R heterophase in the 1896's. Therefore using a combination of

~~to~~ Review the test cross for role of F+. F+ x F- combinations may be more futile

A W1876 x 58-161 EMS Male D(0) D(smr) ! Appl. to EMS 1a, 1b
 ca 15% ++ - - if W1177 x 58-161

B W1802 " +++ - -

as before, 1802 and 58-161 are similar x W1177

Repeat x 1177 also! see 915.

A	1896	58-161	x	W1177	D(0)	D(smr)
B	++	++	x	1876	++	+++ } i.e. Hf behaves as F+
C	1802	1876	x	1876	+++	- in 1177 it is not.

3/19 D 58-161 x W1177 + 3 Yield generally
 " W1876 ++ = low. Brass rupturing.
 C W1802 x W1876 +++

3/22 D D(0) D(smr) D(smr)
 E ++ 55/49 20 Note infutability of C
 " +++ 56R/200 - in smr as expected.

Proportion of breakthrough / smr segments is evidently
 greater for D than E. F+ x F- S^R differs from F+ x F+ S^R.

This is consistent with the concept of "relative potency" of BT and TL
 whereby BMF+ x TL is actually BMF-!

equivalent of parents in
 $F_+ \times F_-$

915-

- A. W1802 ($\beta M F_-$) \times W1876 (F_+)
B. ~~W~~ 58-161 (F_+) \times W1177 (F_-) \pm son (A_+ ; B_-)
C. 58-161 (F_+) \times W1876 (F_+) ~~815a~~

also see Guthrie, 1947.

915a was conducted by Mrs. D. C. Costing. It appears to show that W1177 F_+ behaves like filial W1177! Repeat and of other F_+ strains.

note
near
equal

		M EMS Mal	D10) $\xrightarrow{\text{Rep}}$	bac	14 al
A	58-161 x	w677	- > +	244:20-	20-:5+
B	"	w1177	- > +	28+18 !	40-6+
C	"	w1817	+ > -	50% -	8-62+
D	"	w1876	+ > -	5% -	<100% -
E	"	w1896	+ ≥ -	61+6- 10+4- 23+39-	ca 10% -
\oplus	\cong	1368 " 1368	1896 627		

∴ aberrant behavior of filial TLB- stocks is entirely explained by their F+ character !! Restability of segregation ratios is fully explained.

Is phenotypic modification of TL F+ parent possible (by acetars?)

See 915a for quantitative data here qualitatively confirmed.

A	W1678 x W1607	Lac	Mal	$\xrightarrow{\text{Lac/Mal D/0}}$, EMS Lac or Mal.
		$\xrightarrow{\text{Lac/Mal D/0}}$	$\xrightarrow{\text{Lac/Mal D/0}}$	no effect of F+
B	" W1875	?	++	$\xrightarrow{\text{Lac/Mal D/0}}$!
		?	++	on lac segregation caused by <u>influence</u> ? caused by <u>influence</u> ?
C	" W1177	?	?	ca 20% M+
		?	?	also mostly SR
D	" W1876	?	?	6M+ 12-
		?	?	also mostly SR
E	W1687	1607	??	??
		1875	??	??
F	"	1177	??	??
		1876	??	??

J. 58-161 x W1177F+: 902 D 12 Mal: 13+ : 13- ! Partial effect?
 K. 14 Lac: ca 90% +
 L. 17 Mal: ca 35- 58+ minimal effect!
 # numerous sectored cols.

M 1802 D12 ca 90% Lac +
 N 0 D12 " 1 Mal - 2 M+ low fecundity!
 D12 D11 "
 Restreak D17 to verify purity.
 Repeat D12.

A-B. Lac segregation similar in W1678 x W1607 - 1875

C-D " " " " " ; Mal may? be increased in x F+ ?

C-D should be repeated.

C Lac Mal Mtl
 ca 10% + 4+ 1- 32+ 42-

D 2+ 10- 2- ✓

Thus confirms:
 1) 1802 \cong 58-161 x 1177
 2) 1895 x 1177, 1896 \cong 58-161 x 1177

Repeat ^{S-0} in single cols.

		#	% Lac	Mal	
58-161	D17 - 1	-	= +		\therefore D17 result above probably due to admixture
1802	D17 - 1	-	+ > -		
161	D12	-	+		
1802	D12	-	ca 30% other - ca = +, -		D12 should also be repurified and checked
		-	ca 30% Mal -		

Conclusions: in following crosses
the lac and Mel markers follow the parent
indicated first:

915
sum

x		F (by futility)	
W1177, W677	58-161	- +2	
58-161	W-1876, W1896 (also W477, etc.)	+2 +3	
W1802	W1876	- +3	
W1607	W1678	- +5	
W1875	W1678	+2 +5	
W1177	W1688	- +5	
W1876	W1678	+3 +5	
W1687	W1875	+1 +2	
W1687	W1876	+1 +3	
W1177	W1687	- +1 near infit.	
W1607	W1895	- +10	
W1177	"	- "	
W1875	"	+2 "	
W1876	"	+3 "	
892C	W67	W1649	± +..
	W1177	W67	- ±

See 921
921, 915 d. 916 a.

Hfr.

916

2/27/52

"Hfr" received again from Cavalli ca. 2/24/52, after retests on W1033 showed no Hfr activity. Store as W1895.

- a) Platings of W1895 at 10^8 and 10^6 per plate, in comparison with 58-161 showed 100-1000x as many prototrophs.
- Result: b) Effect was same \times W1177, \times W1876. \therefore does not depend on F- opposition;
- c) In one experiment, aerated Hfr was still F+ (same yield vs. 1177, 1876) but not highly fusible as Hfr. Control showed un-aerated Hfr still ++ fusible.
- d) 1895 dil. \times W1607, 1678 \rightarrow few prototrophs! Repeat
- e) 1895 of. A. showed Hfr \times W1177 in aeration
also Hfr \times 1876 does not accord with c).

2/10/52 (v6). 1895 - 1895A. \times 1678. Showed Hfr from dilute plating, but A $++$
 \times 1177 A $++$ - $+++$

This may reflect a partial F- phenocopy effect of aeration.

g. Grow W1895 in broth with W1607, W1177, W1876. Strain out, recover, and test by replica plating

A. 1895/1607.	1895 12 cols. Hfr	\times W1177	F or Hfr?
	1607 2 " "		= 916 G-2

C. 1895 / 1177	\rightarrow 11 cols	{ Hfr \times W1177.
1876.	" "	\therefore No back-dominance

B. 1177 / 1895.	1177: 8 cols. +Hfr? \times 58-161
1876.	1876: 11 cols. No cols \times 58-161

Is this W1177 F- Hfr? Results after isolation. Both: No cols \times W1607.
= 916 G-1

(These mixtures should be repeated)

W1607 / 1895 = 916 G-2

3/11/52 M. Recitation: results.

	cone.	1177	1876
1895		+++	+++
1895A	+	++	

dil.	1177	1876	+ F gal.
	+	++	EMSLac + 10
	1177- $\frac{1}{3}$ Lac	1876- $\frac{1}{3}$ Lac	for seg. 1

streakout
on NDA.
By applying
ca 20/20 all Hfr!
∴ Again, there seems to be ① an incomplete effect of auxotaxis
on the F+ character and ② a depression of Hfr in compatible crosses.

Note absence of F+ effect of W1876 x 1895 of 58-161

3/12 i. Report, using "moderate dilution"; ca 10^8 cells 1895 or 1895A applied

	1177	1876	Normal effect.
1895	+++	++	Appl. to EMS lac.
1895A	++	++	Lac-: + Estim.
	1 3:1		∴ again,
	2 2:1		
	3 2-3:1	W1895 x Hfr F+ shows no	
	4 3:1	modification of seg. ratio!	

1607/1895

916 g1" 1-2. Both gave seemingly very high yields with W1876, - with W1177.
Same question arose with W1177/1895. See above. $g1 = 916 \text{ g2}$.
W1875 tester was day-old. Today's batch!

916 g1	x 1607 -	closer comparison required!
	x 58-161 ++	
	x 1875 +++	
dil.	x 58-161 29	as half-plates:
1177"	x 58-161 10.	916 g1 } x W1875 } -++ !
		w 1177 }
P		
for close comparison		
they were		
very similar		
3/21/52		
		916 g2 } x W1876 } +++
		w 1607 }
		do 1895 x W1876 less fusible than 1607 x 1876?

M Repeat transfer of F+ from W1895: Grow with 1607, W1177 in Petri-dish.
Extract on EMB agar. Pool 40+ colonies:

C W1607/1895 / x 1177 - ∴ again Hfr does not transfer F+!
D W1177 / x 1607 -

① Is F+ found in Hfr? ② Is it absent? Test pooled prototrophs from
1895 x ~~Hfr~~ 916 g1 ~~W1876~~ Y10

3/11/52. h. aeration of W1895 → ~~had~~ a partial effect on F+ Hfr.
In shake plates, 1895A on nutrient agar gave 20% all Hfr.
1895 gave normal lac, Mal- ratios $\times 1177$, or 1876

- i. Similar result.
- j. W1607/1895 W1177/1895 Neither were modified, either \approx F+ \approx Hfr.
- k. Similar result. → l. do. But 916a as half plate test was -.
(presumably incongruous?)
- m. Retrial of j., via son agar, for F+ from W1895.
Both W1607, W1177 (proto-galactosidase) remained F-.
- 3/16 n. Retrial of m. $\frac{1607}{1177}$ → Remain F-, with ++++, +++ \times F+ features.
- o. 1876/1895. ✓OK. 1876 remains F+.
- p. Retrial m.: A ~~1607~~ $\frac{1177}{1895}$ B $\frac{1607}{1177}$ C $\frac{1607}{1033}$ D $\frac{1607}{1895}$
 $\begin{array}{ll} 1607 & 1875 \\ +++ & ++ \\ ++ & + \\ ++ & + \end{array}$ $\begin{array}{l} \text{Results} \\ [1607]! - \text{do this come?} \\ A) + \end{array}$
 $\begin{array}{l} \text{A) proved to be unique} \\ \text{presumably reduced 1895} \end{array}$
- q. Is 1895 more futile than 1607 \times 1876? Kinetics study may be required.
Hfr does not more highly futile Test 2.
about = Test 2

R.	Repeat p	A $\frac{1177}{1895}$	$\times 1607$	$\times 1875$
		A 98-161	+++	++
		B 1033	+++	++
		C 1895	-	+++

∴, as before, Hfr does not produce F+, but W1033 does.

3/8/52.

J. Colwell 1946 J. Bact. 52: 417.

(D/L) + MNQ (from 1% solution in acetone). Strain K12.

1	0	++
2	.0008%	+
3	.0005	+
4	.001	+
5	.002	-

A. Strain out on nutrient agar. No "petites" observed.

B. Re-strain. No petites ". #5 was sterile.
Critical concentration may be between 4 and 5.

C.	1. 0 2. .001 % 3. .0015 4. .002	A) 24h B) 48h		A) No petites " " Few surv. pit?	B no petites sterile
		+++	+++		
		-	-		

3/19/52 Colwell's observations are not confirmed with K-12! Write up

3/27/52 Repeat with Colwell's # II strain = 776-1763 = W1939

	24h	colonies	48h.	colonies
1. 0	++	++	+++	-
2. .0005	+	++	++	-
3. .001	-	++	-	mostly +, some dw? (sterile)
4. .002	-	some dw?	-	+ 1 dw? (practically sterile)

"dw" grew out to full size.

Repeat 4/21/52

colonies:

	24	48h
1. 0	++	+++
2. .001	-	++
3. .0015	-	-
4. .002	-	-

one small spot gave ++ on re-strain.

Colwell specifies previous transform minimal. Try this with W1939 A (single col.)
no lucks.

September 16, 1952.

- W1939A
- 9/13. Colwell sent *Escherichia coli* strains: #1 = original *coli* transferred on minimal medium. #2, 3, 4 = "SCV" selected with guanine.
- 9/14-15. Characterization of SCV verified. #1 grew promptly to give large colonies on NGA. #2-4 gave barely visible colonies in 24 hours. By 48 hours, ca. 2 mm colonies.
- 9/15. inoculate from plates to D(0); Penassay:
- 9/16. All cultures grew very well, #1 perhaps slightly denser by D(0) #1 grew more poorly than others! for further work, use 1 and 2 only.

9/16. Inoculate 917-1 and -2 from Penassay to

		2 days
D(0) 1/9.	++	-
Penassay	+++	++
NSB	++	+
NSB + glucose	+++ acid agglut	++
D(0) Agar	++ ^{2 days}	+
NS Agar	+++	+
EMB Cde	+++	+
EMB Lac	+++	+

Also, mix 1 and 2
ca 1:5 and
streak out

Growth on D(0) comparable to NB, NA. . . not likely single tryptophane requirement. In his paper, Colwell refers to MacLeod's "synthetic medium" but does not specify whether HC was added. In experiments with W1571 (HLByea) MNG .002% was lytic in standing tubes. Guanine was lytic only in acetone. Usefulness in place of penicillin still to be verified.

Walsman strains

2 W1906

918

March 10, 1952.

- A. W1177 + W1906 in Petri dish; streak out on EMBS Yalson. Walsman phase with agar.
 Pick single colonies for F + test. x W1607. 5 singles - | 6 singles
 Isolate single colonies
 $= 902 D 35$
 pool xt ++. 3 +
 3 - | pool ++
 (3)
- B. Crosses: auxotrophs.
- | | | | | | | |
|-------|---|------|---------------------------------------|------|---------------------------------|----|
| W1907 | - | 1902 | - | 1909 | control platings | -- |
| 1907 | x | 1909 | - | | | |
| 1907 | x | 1607 | - | | | |
| | | 1875 | - | | | |
| 1909 | x | 1177 | + + ^{contaminant} | etc? | Replc to EMBS lac: poor growth. | |
| | | 1876 | - | | prob. contains. | |
- C. W1846
- | | | | | | | |
|---|---|------|------|----|--|--|
| " | x | 1177 | - | | | |
| A | " | x | 1876 | - | | |
| B | " | x | 1607 | 4? | | |
| " | " | x | 1875 | 1 | | |
- $\{ \rightarrow$ all Lac- prototrophs. Fertility suggested.

D. Walsman. SRP x

1177 { rare Malt
 1876 no - each!
 1607
 1875 } This would have been classified as
 doubtful futile to be retested.

E-F (W1852; W1909)

		E	F
1	1607	-	?
2	1875 1876	-	M "
3	1177	1	-
4	1876	-	"
5	1808	-	"
6	1678	-	"

E3 were others did not

\therefore pure TS strains may be detectably futile, thus not (yet)

1177 + 1909 : grow esp and together : -, -

1895 * ?

March 28, 1952

1.	W1852	\times	W1177	37°	-	
2	"	"		+ part.	-	
3	"	"		+ part.	-	ca 20 cols. eventually
4	"	\times	W1895		-	
5	W1846	\times	W1895	+ phage-ridden		
6	W1907	\times	"	"		heavy background lysis
7	W1908	\times	"	ca 1000		

∴ Hfr does allow crossing of wg 35 \times wg 1., but φ^R tester should be used
 EML is working on the extraction of Walsoken phage and transfer to
wg - 1.

ca. 4/10/52. EML noted Wals. to be sucr + - ± at EMIB suc.

Comparison of W1906; W1811; W1852; K12 shows the first 3 to be
 alike, strengthening conclusion of origin from wg 35. Cf. streak for
 cross-reaction (H?) of wg - 1 \times wg - 35.

4/15/52. Strains received from Maes: K1t-p A) K-1t h2 B)

Grow together in Petri-dish 3h. Plate ca. 3 ml purple

W1177 - A No prototrophs

W1177 - B "

A - B Minute colonies; background growth (synthesis?)

A - 1-5 " "

B. " "

? Was K1t-p or -h2 ever properly crossed with ~~#~~wg 1?
 as claimed by Maes? WG-35 behaves in my hands throughout as
 a nearly sterile wg., but there is some likelihood of crossing
 with other wg's.

March 10, 1952.

	$W1903 (= W1688 S^R)$	\times	EMS lac	Picks
A	W1325	40-50/plate	ca 2-:1+	ca 30
B	W1178	5-10/plate	ca 5-:1+	ca 10

Picks lac+ and streak on EMB lac to look for lac_v. ~~+~~

A. 1 lacv \rightarrow lacv Mal-

B. 5 lacv \rightarrow lacv 4 Mal- 114 al+ No Thal_v!

Presumably Thal- are hemizygous and Thal segmented chloroplasts also occurs in these "outcrosses".

c. W478 \times W1876 (for formal statement). EMS lac, Thal. + > -.

EMB poor - diff. mutant? 40 tests - 8? Thal_v.

Recheck from EMS lac.

✓ VP prototroph for
W1927 Wall

4 more? / 40 tests?

Lac Thal

Lac	Thal
+	+
+	-
+	-
+	-
+	+
✓	+
+	+
+	-
✓	+
+	-
?	+
+	-

None of these are useful
for reversion analysis.
cf. Mal states of W478 \times W1177

Should try W1178 \times Y10F+

From EMS

From EMB

March 14, 1952.

58+161 + W1177. Contacted and separated.

ERL micromanipul.

3/12/52 Growing cells contacted on Nuts. agar. Pools assayed x W1607

		count	F
1.	on needle	5 min	-
2.	colonies mixed	2 hours.	-
3	control.	50	-
{ 4		50	-
{ 6	Y → << 30"	42	-
5	control	88	-
9) 10"	57	-
11	near	30 min	-

3/26/52.

1	-
2	-
3	-
4	++
5	-
6	-
7	-
8	-
9	-
10	++
1875	++

7/21/52

#	no cells positioned	no after 3 hrs.	no. on plate (5-6 hrs)	hrs. in contact	has. in contact
1	3	8, 20	13	30	4 $\frac{1}{2}$ - 2 *
2	2	32	16	crowded	1 $\frac{1}{2}$ - 2
3	2 (1 dead?)	17	14	0	4
4	3	16, 16, 16	27	crowded	0 - 3 $\frac{1}{4}$ *
5	2	16, 16	27	65	1 $\frac{1}{2}$ - 1 **
6	2	38	14	crowded	1 $\frac{1}{2}$ - 2
7	3	27, 13	7	"	0 - 2
8	2	60 (4 hrs.)	42	53	1 $\frac{1}{2}$ - 2
9	2	14, 12	17	crowded	1 $\frac{1}{4}$ - 1
10	4	15, 13	35	50	1 - 2

Dividing cells from 58-161 and W1177 placed near each other, grown at room temp. 2-4 hrs before the microcolonies coalesced. Hrs. in contact is the time from the first observance of coalescence until the mixed col. was picked up and plated on E.A.O. inc.

* When >1 cell was present originally, it was not definite which coalescence brought them together.

** #5 2 microcolonies were mixed with each other after 4 hrs. when each was about 30 cells.

3/17/52

A W1895 × W477
 B " " × W677
 C " " × W1896

Very crowded. Pool and restreak.

A. 12 picked. 2 probable Lac^v. Restreak.(2 Lac^v). → 1 Lac^v = 921A

- ↓
 1 Lac-M-
 2 Lac+TLD_r
 3 Lac-M-
 4 Lac+TLD_r

B. Pool tested for transduction to W1607. All F- by transduction test!

strains in EHB_m

see over

C.	Lac	Mal	Lac	Mal	Potential transductors	see over
1	+	+	+			
2	+	+	+			
3	+	-	+			
4	+	+	+			
5	+	+	+			
6	+	-	+			
7	+	+	+			
8	+	-	+			
9	+	-	+			
10	-	-	Mal			
11	-	-	Mal			
12	-	+	Mal			

Test for transmission of F+ to W1177.

Test exposed W1177 / W1607

921B should be tested for fertility × W1177, W1817

A)

- 1 A1 × A2
 2 A1 × 1177
 3 A1 × 1876

- 3
 2
 16 → 14S^s

∴ A1 behaves like a weak F+

- ++ → lac_m. all -R
 + (140) " 1+R.

A2 ~~behaves~~ like a moderate F+. A1 × A2 not acc'td for (unless F =)Replia A1-1876 { EHB_m lac_m
 A2 × 1876 }

Therefore
 FA2 > 1875
 FA1 < 1876

In these experiments, Hfr behaves like an F-, with F+Hfr not hfr.

921B #1 = Lac +
2-8 Lac -

not necessary for
SRP x 1876
1171

Plot. (W1895 x W677) F states by SRP test

921B

~~3/30/52~~ 4/3/52

#1 lac+ 2-8 are lac-. No 1 ml lysis assay to prepare SRP x 1177, 1876

	1177	1876	Second test		
1	-	0	+	46	1177 1817 control
2	-	0	+	22	
3	-	0	+	47	
4	-	0	+	101	
5	-	7	+	42	0 370 0
6	-	0	+	38	
7	-	4	+	109	0 108 4 *
8	-	0	+	77	pres. ^{susceptants}

Can these be re-F+'d?

B1-W1876	11-1	1177 236	1817 27	control 54
	11-2	128	7	21
	1	ca 150	5	0
	2	21	2	0

Hfr x F- \rightarrow F- prototrophs which can be transduced F+.

✓ on EMB Mal.

F+ (wg_x) crosses

923

W1177 x

1876

1678

C 11	-	++	+++
C 12	-	+++	+++
C 14	-	+++	+++
1875	+	+	++
1678	+	/	+++
1607		++	

d 11

d12

d14

C 11	3	c12	+	c14	+
1607	2	1607	+		+
1875	++	1875	++		+++
1678	++	1678	++		++

C 11-12-14 have evidently became again F-

d " " " have retained F+ character, but are much weaker F+ than
W1876. D12 (see above 915 a) may have became mixed F+/F-

March 26, 1952.

R = rutin Q = quercitin.

		Cells from 24 hr Pen assay.	SD-161	and W1895 1 ml / 10 Pen sup 115 PM - 9 PM x W1177
1. *	SD-161	Sup.	+	
2.	"	(Illuminated Hamonia green) 60 sec.	++(?) Moreover	
3.	"	Rutin $\frac{1}{2} \times 10^{-4}$ = .05 mg/ml	++ ✓?	
4.	"	Quercitin "	++	
5.	1895	-	++++	
6.	"	Rutin $\frac{1}{4}$	++++	
7.	"	Rutin $\frac{1}{2}$	++++	

B = plated with .25 mg rutin per plate : 1 +
5 ++++

* tube broken! Use residual cells from moribund tube.
incubating.

Rutin had no effect!

March 27, 1952

1	58-161	
2	"	Light 60s.
3	"	Reticul. 1/4
4	"	green 1/4
5	W1895	
6	"	Reticul. 1/4

2 11/10	11 AM - 2 30 PM
+ (19)	1B (reticul.) + 65
+	1C + (15)
+	
+	
+++	5B +++
+++-	

(5C: illum Harronia-glass)

Reberle is control for strain effect of frutin on 58-161 x W1177; light as 1895.

58-161, W1177 grown in dark (red glass).

1. 58-161, W1177 grown in dark (red glass).
2. Fluoresc lamps 10 mm.
3. Harronia (through glass) "
4. 58-161 x W1177 grown without dark precautions

no significant
effect of light.

1:	56, 57
2:	64, 61
3:	57, 85
4:	98, 74

F+ Hormone from W 1895

925

March 26, 1952.

A Cross W1895 with W1607. Plate on D(0), D(sun), EMS lac. $\times Y10$

B. Control system, grown separately

C. " components. 1 1895 \times ~~Y10~~ Y10
2 ~~W1607~~ \times Y10

D streak out $\frac{1607}{1895}$. ca % % lac +
15%

A. D(0) +++ \xrightarrow{R} EMS lac sun 4 SRP. later ca 20 addnl. SRP,
D(sun) No SRP. some lac-!
3 days. ca 100!

B. D(0) +++ \xrightarrow{R} EMS lac sun 925A1 streakout + checks
D(sun) 0 500 + 1 ? 6 SRP, lac, -
EMS lac all +

C1 D(0) +++ \xrightarrow{R} EMS lac sun 0.
C2

Review of possibility of intercalary crossing of W1607 \times W1895, these data provide no support for a hormonal control of F+ grade in a situation where F+ transduction does not occur. See 928

May be crossing probably occurs on ^{EMS} sun plates.